CODON-OPTIMIZED TRANSGENE FOR THE TREATMENT OF PROGRESSIVE FAMILIAR INTRAHEPATIC CHOLESTASIS TYPE 3 (PFIC3)

FIELD OF THE INVENTION

[0001] The present disclosure relates to gene therapy vector for use in the treatment of progressive familiar intrahepatic cholestasis type 3. More specifically, the present invention relates to an adeno-associated virus vector comprising codon-optimized sequence encoding for the MDR3 isoform A for the treatment of PFIC3.

BACKGROUND ART

[0002] Progressive familial intrahepatic cholestasis type 3 (PFIC3) is a genetic disease associated with mutations in adenosine triphosphate-binding cassette, subfamily B, 4 (ABCB4) gene, coding for multidrug resistance protein 3 (MDR3) (Jacquemin E. Clin Res Hepatol Gastroenterol. 2012; 36 Suppl 1:S26-35). This protein, which is expressed predominantly in the canalicular membrane of hepatocytes, is a floppase involved in the translocation of phosphatidylcholine from the hepatocyte membrane to the bile. Phosphatidylcholine is necessary to neutralize the toxicity of bile salts through the generation of mixed micelles. Mutations in ABCB4 gene prevent proper micelle formation, resulting in bile canaliculi and biliary epithelium injury, leading to cholestasis (Jacquemin E. et al. Gastroenterology. 2001; 120:1448-1458).

[0003] There is currently no cure for PFIC3, and therefore the unmet medical need is very high. Although ursodeoxycholic acid (UDCA) therapy may ameliorate symptoms in some patients, outside of liver transplant there is currently no curing treatment for PFIC3. Surgical intervention in the form of biliary diversion improves patient outcomes. However, post-surgical complications such as infections and issues with stoma bags impact patients' quality of life, while the risk of cirrhosis and liver cancer still remains. Liver transplants are an effective treatment, but carry with them the risks involved with such a complicated procedure as well as a chance of re-emergence of the condition (van der Woerd W L et al. World J gastroenterol. 2017; 23(5):763-775).

[0004] Gene therapy correcting the defective gene responsible for disease development is a promising treatment for a number of diseases. However, the technique remains still under study. Stable integration and expression of ABCB4 in liver cells to treat or prevent PFIC3 is disclosed in WO2015/139093. However, these integrating vector systems present a main disadvantage that is their potential risk of causing insertional mutagenesis. RNA therapy to treat a liver condition such as progressive familial intrahepatic cholestatsis 3 (PFIC3) using various potential therapeutic genes including ABCB4 was only suggested in WO2017/100551. Thus, there is still a need to develop gene therapy methods which avoid insertional mutagenesis and meanwhile allow stable and long term transgene expression. It is described herein a PFIC3 gene therapy with codon-optimized sequences encoding MDR3 iso form A that allows successful high expression in cholestatic liver so as to revert the cause of the toxicity in the bile of affected patients.

SUMMARY OF THE INVENTION

[0005] Surprisingly, the inventors found that contrary to the wild type MDR3 iso form A, the codon optimized sequence of MDR3 isoform A when administered in vivo showed an efficient expression specifically in the canalicular membranes of hepatocytes. In Abcb4$^{-/-}$ knock-out mice which reproduce most of PFIC3 symptoms, administration of AAV encoding codon optimized versions of MDR3 iso form A achieve a long-term therapeutic effect such as significant restoration of PFIC3 serum biomarker levels, decrease of liver and spleen size, increase of bile phosphatidylcholine and correction of the liver morphology abnormalities.

[0006] A first aspect of the present disclosure thus relates to a nucleic acid construct comprising a transgene encoding MDR3 isoform A, said transgene being represented by SEQ ID NO: 1 or a sequence having at least 90% of identity with SEQ ID NO: 1.

[0007] In specific embodiments, said nucleic acid construct further comprises a promoter which initiates transgene expression upon introduction into a host cell, preferably a liver specific promoter, more preferably an alpha-1-antitrypsin promoter or a bile salt-inducible promoter. In specific embodiments, said vector further comprises a polyadenylation signal sequence, for example a synthetic polyadenylation signal sequence having sequence SEQ ID NO: 3.

[0008] In specific embodiments, said nucleic acid construct further comprises a 5'ITR and a 3'ITR sequences, preferably a 5'ITR and a 3'ITR sequences of adeno-associated virus (AAV), notably a 5'ITR and a 3'ITR sequences from the AAV2 serotype.

[0009] In more specific embodiments, said nucleic acid construct comprises or consists of a nucleic acid sequence SEQ ID NO: 4 or a nucleic acid sequence having at least 90% of identity with SEQ ID NO: 4.

[0010] In another aspect, said nucleic acid construct is comprised in an expression vector, preferably a viral vector, more preferably an AAV vector.

[0011] Another aspect of the present disclosure relates to a viral particle comprising a nucleic acid construct or an expression vector of the invention, and preferably comprising capsid proteins of adeno-associated virus such as capsid proteins selected from the group consisting of: AAV3 type 3A, AAV3 type 3B, NP40, NP59, NP84, LK03, AAV3-ST, Anc80 and AAV8 serotype.

[0012] Another aspect of the present disclosure relates to a host cell comprising the nucleic acid construct or the expression vector of the invention, or a host cell transduced with a viral particle of the invention.

[0013] Another aspect of the present disclosure relates to a pharmaceutical composition comprising the nucleic acid construct, expression vector, host cell, or viral particle of the invention, in combination with one or more pharmaceutical acceptable excipient, diluent or carrier, optionally comprising other active ingredients.

[0014] The invention also relates to a product of the invention for use as a medicament, such as the prevention and/or the treatment of progressive familial intrahepatic cholestasis type 3 in a subject in need thereof. In a specific embodiment, the subject is a neonate, an infant, a child or an adult, preferably a neonate, an infant or a child, more preferably a neonate or an infant.

[0015] Also disclosed herein is a process for producing viral particles as described above, comprising the steps of: a) culturing a host cell as described above in a culture medium, and b) harvesting the viral particles from the cell culture supernatant and/or inside the cells.